AD_____

Award Number: DAMD17-01-1-0593

TITLE: Relative Contribution of Ornithine Decarboxylase (ODC) Versus S-adenosylmethionine Decarboxylase (SAMDC) to Human Breast Cancer Progression and Development

PRINCIPAL INVESTIGATOR: Andrea Manni, M.D.

CONTRACTING ORGANIZATION: Pennsylvania State University
College of Medicine
Hershey, Pennsylvania 17033

REPORT DATE: July 2002

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Final (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Relative Contribution of Ornithine Decarboxylase (ODC) Versus S-adenosylmethionine Decarboxylase (SAMDC) to Human Breast Cancer Progression and Development

5. FUNDING NUMBERS
DAMD17-01-1-0593

6. AUTHOR(S)
Andrea Manni, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Pennsylvania State University
College of Medicine
Hershey, Pennsylvania 17033

E-Mail: amanni@psu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

Although polyamines (PA) play an important role in breast cancer phenotype, the relative contribution of the two PA biosynthetic enzymes, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC) is not known. Our data show that overexpression of SAMDC in MCF-7 breast cancer cells leads to a more benign biologic behavior characterized by decreased invasiveness *in vitro* and reduced tumorigenicity in nude mice. These effects may be mediated by the compensatory downregulation of ODC leading to reduced cellular putrescine and spermidine contents. In contrast, in experiments conducted in MDA-MB-435 and MDA-MB-231 breast cancer cells, we have observed that ODC adversely affects breast cancer phenotype and, most importantly, contributes to the development of distant metastasis. In the aggregate, our data point to ODC as an attractive target in the adjuvant therapy of breast cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
polyamines, ornithine decarboxylase, S-adenosylmethionine decarboxylase, breast cancer metastasis	9
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction:

Considerable evidence indicates that polyamines (putrescine, spermidine, and spermine) play and important role in breast cancer biology (1). In particular, it appears that they may contribute to mammary carcinogenesis (2,3) as well as breast cancer progression to a more aggressive and metastatic hormone-independent phenotype (4). However, the relative contribution of the two polyamine biosynthetic enzymes, namely ornithine decarboxylase (which primarily controls the synthesis of the diamine putrescine) and S-adenosylmethionine decarboxylase (which mediates the production of the more distal polyamines spermidine and spermine) is not known. Therefore, our concept proposal planned to address this issue using the MCF-7 breast cancer cell line as well as a transgenic approach.

Body:

In contrast to the large body of evidence supporting an important role of ODC, very limited information is available on the possible influence of SAMDC on breast cancer phenotype. Therefore, our first priority was to investigate the effects of this enzyme on breast cancer behavior using SAMDC overexpressing MCF-7 breast cancer cells which were generated in our laboratory (5). Since SAMDC promotes the formation of the distal polyamines spermidine and spermine, we hypothesized that induction of its overexpression would adversely affect breast cancer phenotype. Indeed, some preliminary evidence generated in a previous publication (5) from our group suggested that SAMDC-MCF-7 cells were more clonogenic and bypassed the need of serum and estrogens for growth in soft agar. More detailed analysis of the properties of these cells, however (as described in our attached publication [6]), did not confirm these initial findings and indicated instead that SAMDC overexpression conferred a more benign phenotype. As described in detail in our publication (6), SAMDC overexpressing MCF-7 cells manifested reduced invasiveness in matrigel and were less tumorigenic in nude mice. Furthermore, the growth of established xenografts was slower. In addition, control and SAMDC overexpressing cells did not differ with regard to sensitivity to estrogens and anti-estrogens, thus indicating that activation of this enzyme did not lead to hormone independence. In

retrospect, these results are not surprising since SAMDC overexpression is associated with a compensatory downregulation of ODC and activation of SSAT which results in near total suppression of putrescine and an approximately 50% reduction in cellular spermidine level (5,6). Cellular spermine content is increased *in vitro* but is not different from control *in vivo* (6). Therefore, this polyamine profile is similar to that induced by inhibition of ODC with alpha-difluoromethylornithine, a drug which inhibits breast cancer cell proliferation and reduces invasiveness and metastasis. SAMDC overexpression in our experimental system did not modify activation of MAPK, or STAT signaling in response to EGF administration (6). In contrast, we have observed, in a different experimental system (the immortalized MCF-10A human breast epithelial cell line), that ODC overexpression increases phosphorylation of MAPK (2,3) and tyrosine phosphorylation of STAT-3 (unpublished observations). Unfortunately, we have been unable to extend the characterization of our ODC overexpressing MCF-7 cells since, in order to maintain elevated levels of ODC, we needed to chronically select the cells with DFMO which was found to have effects unrelated to the polyamine pathway. This was surprising to us since DFMO selection has been extensively used in the literature to induce ODC overexpression. Nevertheless, these extraneous effects of DFMO made our experimental system unusable. Therefore, we have used a different experimental approach to address the role of ODC in breast cancer progression. In recently published work, we have observed that inhibition of ODC activity with DFMO reduced *in vitro* invasive features of aggressive hormone independent MDA-MB-435 and MDA-MB-231 breast cancer cells and, most importantly, nearly totally abolished pulmonary metastasis from MDA-MB-435 breast cancer xenografts in nude mice (7). In summary, our up-to-date results indicate that ODC does, indeed, play a major role in breast cancer progression, contributing in a major way to development of metastasis, while SAMDC (at least when overexpressed) favorably influences breast cancer behavior.

We have placed considerable effort in trying to generate transgenic mice with targeted overexpression of ODC to the mammary gland. We obtained from Dr. Dean Selcher at UCSF, CA,

FVB-MMTV-Tet-Ta mice which are homozygous for the Tet transactivator driven by the MMTV promoter. Tet-Ta binds to the tetracycline responsive element (TRE) and activates transcription of the target gene (ODC in this case) in the absence of doxycycline (tet/off). In order to create mice overexpressing ODC in the mammary gland, the above mice were crossed to homozygous C57BL/6 transgenic mice carrying ODC under the control of a minimal CMV promoter attached to a multimerized TRE (obtained from Dr. Tom G. O'Brien, Lankenau Medical Center, Wynnewood, PA). Although we were able to generate mice with the appropriate phenotype, we were unable to detect ODC overexpression in their mammary gland. We are now trying a different promoter, C3(1) which has been reported to be successful in inducing gene expression in the mammary gland. These studies are currently funded by a Dean's Feasibility Grant at our institution.

Key Research Accomplishments:

- SAMDC overexpression reduces invasiveness of breast cancer cells.
- SAMDC overexpression reduces tumorigenicity in nude mice of MCF-7 breast cancer cells and retards the growth of established tumors.
- SAMDC overexpression does not influence the hormone responsiveness of MCF-7 breast cancer cells.
- ODC mediates *in vitro* invasiveness of hormone independent MDA-MB-435 and MDA-MB-231 breast cancer cells.
- ODC mediates the development of pulmonary metastasis from MDA-MB-435 human breast cancer xenografts in nude mice.

Reportable Outcomes:

Manni, A., Fischer, S., Franks, M., Washington, S., DeArment, R., Griffith, J., Demers, L., Verderame, M., Leiby, B., Mauger, D. S-adenosylmethionine decarboxylase (SAMDC) overexpression reduces invasiveness and tumorigenicity in nude mice of MCF-7 breast cancer cells. Int J Oncology, 19:317-323,2001.

Manni A, Washington S, Griffith JW, Verderame MF, Mauger D, Demers LM, Samant RS, Welch DR. Influence of polyamines on *in vitro* and *in vivo* features of aggressive and metastatic behavior by human breast cancer cells. Clin Experimental Metastasis 19:95-105, 2002.

Manni, A., Fischer, S., Franks, M., Washington, S., DeArment, R., Griffith, J., Demers, L., Verderame, M., Leiby, B., Mauger, D. S-adenosylmethionine decarboxylase (SAMDC) overexpression reduces invasiveness and tumorigenicity in nude mice of MCF-7 breast cancer cells. The Endocrine Society's 83rd Annual Meeting, June 20-23, 2001, Denver, CO, abstract #P2-612.

Manni, A., Washington, S., Griffith, J., Verderame, M.F., Mauger, D., Demers, L.M. Polyamine involvement in invasion and metastasis by human breast cancer cells. 2001 Breast Cancer Symposium, December 10-12, 2001, San Antonio, Texas, Abstract.

Michael F. Verderame, "Targeted Overexpression of ODC and the ODC Inhibitory Antizyme-1", Dean's Feasibility Grant, July 1, 2002-June 30, 2003, $24,011 (DC).

Conclusions:

The observation that SAMDC overexpression has a favorable influence on breast cancer phenotype has important clinical implications since inhibitors of SAMDC are actually currently being developed as anticancer agents (8). Although found to be effective in reducing proliferation of breast cancer cells, our preliminary data in SAMDC overexpressing MCF-7 cells suggests that the antitumor effects of these compounds may be mitigated by the marked compensatory increase in ODC and putrescine levels induced by these drugs. Therefore, they may best be utilized in combination with inhibitors of ODC activity such as DFMO.

A major finding is that ODC is involved in distant metastasis from breast cancer. If this observation is confirmed in future studies, it would provide a strong rationale for testing the feasibility of inhibitors of ODC such as DFMO in the adjuvant therapy of breast cancer. It is worth noting that

inhibition of polyamine biosynthesis is effective in hormone independent tumors, i.e., those with the most aggressive behavior and for which treatment options are limited.

References

1. Manni A. Role of the polyamine pathway in the natural history of breast cancer. IN: *Contemporary Endocrinology: Endocrinology of Breast Cancer,* A. Manni (ed), Humana Press, Inc., Totowa, NJ, pp. 221-230, 1998.

2. Manni A, Wechter R, Gilmour S, Verderame MF, Mauger D, Demers LM. Ornithine decarboxylase over-expression stimulates mitogen-activated protein kinase and anchorage-independent growth of human breast epithelial cells. Int J Cancer 70:175-182, 1997.

3. Manni A, Wechter R, Verderame M, Mauger D. Cooperativity between the polyamine pathway and HER-2neu in transformation of human mammary epithelial cells in culture: Role of the MAPK pathway. Int J Cancer 76:563-570, 1998.

4. Manni A, Grove R, Kunselman S, Demers L. Involvement of the polyamine pathway in breast cancer progression. Cancer Lett 92:49-57, 1995.

5. Manni A, Badger B, Grove R, Kunselman S, Demers L. Isolation and characterization of human breast cancer cells overexpressing S-adenosylmethionine decarboxylase. Cancer Lett 95:23-28, 1995.

6. Manni, A., Fischer, S., Franks, M., Washington, S., DeArment, R., Griffith, J., Demers, L., Verderame, M., Leiby, B., Mauger, D. S-adenosylmethionine decarboxylase (SAMDC) overexpression reduces invasiveness and tumorigenicity in nude mice of MCF-7 breast cancer cells. Int J Oncology, 19:317-323,2001.

7. Manni A, Washington S, Griffith JW, Verderame MF, Mauger D, Demers LM, Saman RS, Welch DR. Influence of polyamines on *in vitro* and *in vivo* features of aggressive and metastatic behavior by human breast cancer cells. Clin Exptl Metastasis 19:95-105, 2002.

8. Regenass U, Mett H, Stanek J, Muller M, Kramer D, Porter CW. CGP 48664, a new S-adenosylmethionine decarboxylase inhibitor with broad spectrum antiproliferative and antitumor activity. Cancer Res 54:3210-3217, 1994.